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NIST Mixture Interpretation Interlaboratory Study 2005 (MIX05)

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The human identity project team within the Biotechnology Division of the National Institute of Standards and Technology (NIST) is funded by the National Institute of Justice (NIJ) to conduct research that benefits the human identity testing community and to create tools that enable state and local DNA laboratories to be more effective in analyzing DNA. We have conducted a number of interlaboratory studies (see http://www.cstl.nist.gov/biotech/strbase/interlab.htm) over the years to assess consistency in results from multiple laboratories with mixture interpretation (1,2) and DNA quantitation methods (3). In early 2005 an interlaboratory challenge exercise was initiated involving only data interpretation. DNA mixtures representing four different mock sexual assault case scenarios were generated at NIST with multiple STR kits and provided to laboratories as electrophoretic data (ABI 3100 .fsa files are available at http://www.cstl.nist.gov/biotech/strbase/interlab/MIX05.htm). In each case, we provided the "evidence" sample result, which was a mixture of at least one perpetrator and a victim, along with the "victim" reference sample. All data were generated on six different STR kits (Profiler Plus, COfiler, SGM Plus, Identifiler, PowerPlex 16, and PP16 BIO) from the same lot of DNA mixtures. Those labs, including Macintosh-based users, that could not download data from the MIX05 website were shipped CD-ROMs or zip disks.

The MIX05 interlaboratory study was designed (1) to evaluate the current "lay of the land" regarding STR mixture interpretation across the forensic DNA typing community and (2) to aid development of training tools to enable mixture interpretation and reporting. The sample selection process is described here for the samples used in the study. A discussion of MIX05 participants responses will be part of future publications.

References

1) Duewer DL, Kline MC, Redman JW, Newall PJ, Reeder DJ. (2001) NIST Mixed Stain Studies #1 and #2: interlaboratory comparison of DNA quantification practice and short tandem repeat multiplex performance with multiple-source samples. *J. Forensic Sci.* 46: 1199-1210.

2) Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J.M. (2003) NIST mixed stain study 3: DNA quantitation accuracy and its influence on short tandem repeat multiplex signal intensity. *Anal. Chem.* 75: 2463-2469.

3) Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J.M. (2005) Results from the NIST 2004 DNA Quantitation Study. *J. Forensic Sci.*, 50(3): 571-578.

Participant Enrollment

Initial enrollment through announcements and handouts made at the following forensic meetings:
- CODIS User's Group (November 15, 2004)
- Forensic Y User's Group (November 20, 2004)
- SWGDAM (January 18, 2005)

Emails to previous participants in NIST interlab studies such as Mixed Stain Study 3, DNA Quantitation Study 2004

70 labs initially enrolled (28 states, 17 overseas)

A second email push was made in January 2005

Total of 94 labs enrolled by June 2005

Participants in MIX05

(total of **69 labs responded**; listed alphabetically by state)

Alabama Department of Forensic Sciences (Birmingham, AL)
Arkansas State Laboratory (Little Rock, AR)
Scottsdale Police Department (Scottsdale, AZ)
San Diego County Sheriff's Department (San Diego, CA)
California Department of Justice DNA Lab (Richmond, CA)
Orange County Sheriff's Office (Santa Ana, CA)
Colorado Bureau of Investigation (Pueblo, CO)
Colorado Bureau of Investigation (Montrose, CO)
Colorado Bureau of Investigation (Denver, CO)
Connecticut Forensic Lab (Meriden, CT)
Office of Chief Medical Examiner (Wilmington, DE)
Florida Department of Law Enforcement (Jacksonville, FL)
Florida Department of Law Enforcement (Orlando, FL)
Palm Beach County Sheriff's Office (West Palm Beach, FL)
US Army Crime Laboratory (Forest Park, GA)
Indianapolis-Marion County (Indianapolis, IN)
Indiana State Police (Indianapolis, IN)
Sedgwick County Regional Forensic Science Center (Wichita, KS)
Kansas Bureau of Investigation (Topeka, KS)
Kentucky State Police (Frankfort, KY)
Massachusetts State Police Crime Lab (Sudbury, MA)
Baltimore County Police (Towson, MD)
Baltimore City Police Department (Baltimore, MD)
Maryland State Police (Baltimore, MD)
Prince George's County Police Department (Landover, MD)
Minnesota Bureau of Criminal Apprehension (St. Paul, MN)
North Dakota Office of Attorney General (Bismarck, ND)
Human DNA Identification Laboratory (Omaha, NE)
New Mexico Department of Public Services (Santa Fe, NM)
Washoe County Sheriff's Office (Reno, NV)
New Jersey State Police (Hamilton, NJ)
Suffolk County Crime Laboratory (Hauppauge, NY)
Office of Chief Medical Examiner (New York, NY)
Westchester County Forensic Lab (Valhalla, NY)
Miami Valley Regional Crime Lab (Dayton, OH)
Columbus Police Crime Lab (Columbus, OH)
Oklahoma City Police Department (Oklahoma City, OK)
Oregon State Police (Clackamas, OR)
Pennsylvania State Police (Greensburg, PA)
Rhode Island Department of Health (Providence, RI)
South Dakota State Forensic Lab (Pierre, SD)
Harris County Medical Examiner's Office (Houston, TX)
Myriad Genetic Laboratories Inc. (Salt Lake City, UT)
DNA Consulting Associates (Annandale, VA)
Virginia Division of Forensic Sciences (Richmond, VA)
Virginia Division of Forensic Sciences (Roanoke, VA)
Virginia Division of Forensic Sciences (Fairfax, VA)
Virginia Division of Forensic Sciences (Norfolk, VA)
Vermont Forensic Lab (Waterbury, VT)
Washington State Police (Seattle, WA)
Wisconsin Department of Justice (Milwaukee, WI)
Wisconsin State Crime Lab (Madison, WI)

Servicio de Huella Digitales Geneticas (Buenos Aires, ARGENTINA)
Legal Medical Service of Chile (Santiago, CHILE)
Palacky University, Dept Biochemistry (Olomouc, CZECH REPUBLIC)
Department of Forensic Genetics (Copenhagen, DENMARK)
BKA Baden-Wuerttemberg (Stuttgart, GERMANY)
State Criminal Office Saxony (Dresden, GERMANY)
Institute of Forensic Medicine (Budapest, HUNGARY)
Institute of Forensic Sciences (Budapest, HUNGARY)
Department of Medicine and Public Health (Bologna, ITALY)
Institute of Legal Medicine (Pedova, ITALY)
Institute of Legal Medicine (Modema, ITALY)
Department of Anatomy & Pharmacology (Turin, ITALY)
Chemistry Department of Malaysia (Selangor, MALAYSIA)
Institute of Forensic Medicine (Golansk, POLAND)
Instiuto Nacional de Toxicologia (Barcelona, SPAIN)
Instituto Nacional de Toxicologia (Madrid, SPAIN)
Instituto Nacional de Toxicologia (Sevilla, SPAIN)

Sample Design for MIX05

Samples were selected for the MIX05 study based on review of all possible allele combinations from 40 females and 660 males previously examined with the 15 STRs present in the Identifiler kit (see Butler et al. JFS 2003;48(4):908-911). David Duewer from the NIST Analytical Chemistry Division developed a computer program named **Virtual MixtureMaker** to perform these comparisons (output shown below for selected samples). The program will be made available on STRBase: http://www.cstl.nist.gov/biotech/strbase/software.htm. After various allele combinations were selected with a mixture of 1 male and 1 female, mixture ratios were selected to reflect some common casework scenarios. The DNA extracts were mixed in the laboratory and PCR products generated following manufacturer's recommended conditions.

Number of alleles in mixture and number of unique alleles | Number of loci with 1, 2, 3, 4, or 5 alleles | *Not used in sample selection*

CASE #1	Expected Mixture	N_{all}	N_{uniq}	N_1	N_2	N_3	N_4	N_5	AMEL	CSF1PO	FGA	TH01	TPOX	vWA	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	Penta D	Penta E
"Evidence" Mixture	**Sample S**	39	26	**2**	**6**	**5**	**2**	**0**	X,X,X,Y	11,12	19,20,21,22	7,8	8	15,17	17,21,22,25	15,16,17	11	9,10	12,14,15	11,12	10,11,12	12,15,16	13,14	27,28,31.2	9,13,14	5,7,12
3 parts female: 1 part male																										
	Individual Samples	N_{pc}	N_{all}	N_0	N_1	N_2	N_3		AMEL	CSF1PO	FGA	TH01	TPOX	vWA	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	Penta D	Penta E
Female "Victim"	**Sample P**	16	26	0	6	10	0		X,X	11,12	19,21	8	8	17	17,25	15,16	11	9,10	14,15	11	11,12	12,15	13,14	27,31.2	9,14	7,12
Male "Perpetrator"	*not supplied (T)*	16	26	0	6	10	0		X,Y	11,12	20,22	7	8	15,17	21,22	16,17	11	10	12	12	10,11	15,16	13,14	28,31.2	13	5,7

CASE #2	Expected Mixture	N_{all}	N_{uniq}	N_1	N_2	N_3	N_4	N_5	AMEL	CSF1PO	FGA	TH01	TPOX	vWA	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	Penta D	Penta E
"Evidence" Mixture	**Sample B**	55	52	**0**	**1**	**4**	**10**	**0**	X,X,X,Y	7,10,12,13	20,23,24	7,8,**9.3,10**	8,9,10,11	15,16,19	16,17,21,24	15,16	8,11,12,13	8,9,10,11	11,13,14	8,9,12,14	9,10,11,12	12,15,17,18	12,13,14	28,30,31,32.2	9,10,12	12,15,17
1 part female: 3 parts male																										
	Individual Samples	N_{pc}	N_{all}	N_0	N_1	N_2	N_3		AMEL	CSF1PO	FGA	TH01	TPOX	vWA	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	Penta D	Penta E
Female "Victim"	**Sample A**	16	31	0	1	15	0		X,X	12,13	23,24	8,10	8,11	16,19	16,24	15,16	11,12	9,11	11,14	8,9	9,12	12,15	12,14	30,31	10,12	12,17
Male "Perpetrator"	*not supplied (E)*	16	29	0	3	13	0		X,Y	7,10	20,24	7,9.3	9,10	15	17,21	15	8,13	8,10	11,13	12,14	10,11	17,18	13	28,32.2	9	12,15

CASE #3	Expected Mixture	N_{all}	N_{uniq}	N_1	N_2	N_3	N_4	N_5	AMEL	CSF1PO	FGA	TH01	TPOX	vWA	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	Penta D	Penta E
1 part female: 1 part male	**Sample L**	48	37	**0**	**3**	**8**	**4**	**0**	X,X,Y	10,11,13	21,22,23	6,7,8,9	8,9,11	15,16,17	17,18,21,25	16,17,18	11,12	8,9,13	12,15	10,12	10,12,13	12,14,17	11,14.2,15,16.2	27,28,29,30	9,11,12	5,7,12,15
	Individual Samples	N_{pc}	N_{all}	N_0	N_1	N_2	N_3		AMEL	CSF1PO	FGA	TH01	TPOX	vWA	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	Penta D	Penta E
Female "Victim"	**Sample K**	16	28	0	4	12	0		X,X	10,11	21,22	8,9	8,9	15,16	17,18	17,18	12	8,13	15	10,12	12	12,17	11,15	29,30	9,11	5,12
Male "Perpetrator"	*not supplied (O)*	16	29	0	3	13	0		**Y**	11,13	21,23	6,7	9,11	15,17	21,25	16,18	11,12	8,9	12	12	10,13	14,17	14.2,16.2	27,28	11,12	7,15

CASE #4	Expected Mixture	N_{all}	N_{uniq}	N_1	N_2	N_3	N_4	N_5	AMEL	CSF1PO	FGA	TH01	TPOX	vWA	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	Penta D	Penta E
7 parts female: 1 part male	**Sample G**	50	42	**0**	**3**	**7**	**4**	**1**	X,X,X,Y	10,11,12,13	23,24,25	8,9,9.3	**8,9,10,11,12**	15,16,17	17,18,23,24	15,16	9,11,12	9,10,11	9,12,13	12,13	9,11	13,18,19,21	13.2,14,15	27,28,30.2,32	10,13,14	5,14,16
	Individual Samples	N_{pc}	N_{all}	N_0	N_1	N_2	N_3		AMEL	CSF1PO	FGA	TH01	TPOX	vWA	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	Penta D	Penta E
Female "Victim"	**Sample F**	16	27	0	5	11	0		X,X	10,12	23,24	9,3	8,12	16,17	17,18	15,16	9,11	9,11	12,13	13	11	13,19	14	28,32	13,14	5,14
Male "Perpetrator"	*not supplied (J)*	16	31	0	2	13	1		X,Y	11,13	25	8,9	**9,10,11**	15,17	23,24	15,16	12	10,11	9,13	12,13	9,11	18,21	13.2,15	27,30.2	10,13	14,16

Initial Format Requested by Participants

Analysis software requests	STR kit requests	STR kit results used
27 Genotyper Mac	37 ProfilerPlus/COfiler	**34 ProfilerPlus/COfiler**
24 Genotyper NT	16 PowerPlex 16	**10 PowerPlex 16**
23 GeneMapper*ID*	22 Identifiler	**7 PP16 BIO**
6 FMBIO Mac	1 SGM Plus	**5 Identifiler**
2 FMBIO NT	8 FMBIO	**2 SGM Plus**
		1 All ABI kit data
		9 Various combinations

Mixture scenarios to evaluate:
- –Victim is major contributor (Case #1)
- –Perpetrator is major contributor (Case #2)
- –"Balanced" ~1:1 mixture (Case #3)
- –"Extreme" ~1:10 mixture (Case #4)

We supplied female "victim" and mixture "evidence" for each case (along with allelic ladder, pos. & neg. controls)

What We Requested from MIX05 Participants:

1) **Report the results as though they were from a real case** including whether a statistical value would be attached to the results. *Please summarize the perpetrator(s) alleles in each "case" as they might be presented in court—along with an appropriate statistic (if warranted by your laboratory standard operating procedure) and the source of the allele frequencies used to make the calculation.* Please indicate which kit(s) were used to solve each case.

2) **Estimate the ratio for samples present in the evidence mixture** and how this estimate was determined.

3) **Provide a copy of your laboratory mixture interpretation guidelines** and a brief explanation as to why conclusions were reached in each scenario

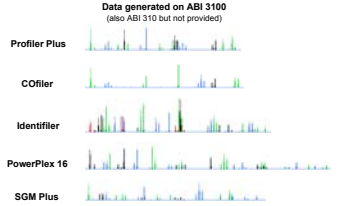
Summary of Responses
- 50 labs made allele calls
- 39 labs estimated ratios
- 29 labs provided stats

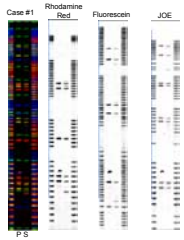
Data Supplied to MIX05 Participants

Materials and Methods
Genomic DNA samples mixed at specific ratios
Commerical Kits: Followed manufacturer protocols with full volume PCR reactions
ABI 3100: 36 cm array, POP-6, 10s@3kV injections, data collection 1.0.1
Data evaluation: GeneScan 3.7 and Genotyper 3.7 or GeneMapper*ID* 3.2

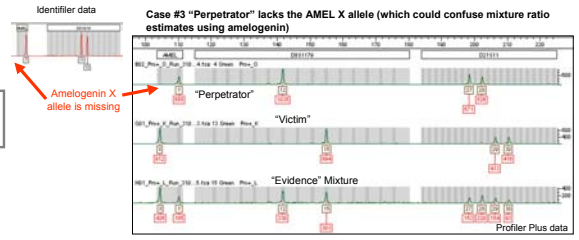
FMBIO data supplied separately—generated in Pennsylvania State Police Lab and Arkansas State Crime Lab using NIST created PCR products

Case 1 "Evidence"Mixture (Sample S)





Decision was made to supply all data for 5 different STR kits (ProPlus, COfiler, Identifiler, PP16, SGM Plus) **to all ABI kit labs**



Comments from Several MIX05 Participants

•I'm anxious to see the paper with everyone's results, it should be interesting. I think it will show **we need some consistent guidelines for mixtures**.

•Thank you for letting us participate in this NIST study. **As always, it is a rewarding educational experience**.

•Thank you for allowing us to participate in the NIST studies. **We always find them very interesting, thought-provoking, and useful**. We look forward to seeing the results from this study and to participating in more studies in the future.

Benefits of MIX05

•Data sets exist with multiple mixture scenarios and a variety of STR kits that can be used for training purposes.

•A wide variety of approaches to mixture interpretation have been applied on the same data set(s) and evaluated as part of a single study.

•**Interpretation guidelines from many laboratories are being compared to one another for the first time in an effort to determine challenges facing future efforts to develop "expert systems" for automated mixture interpretation.**

•We are exploring the challenges of supplying a common data set to a number of forensic laboratories (e.g., if a standard reference data set was ever desired for evaluating expert systems).

Some of the primary benefits we hope to gain from this study include **recommendations for a more uniform approach to mixture interpretation** and training tools to help educate the community.

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•Chris Tomsey & Frank Krist (PA State Police) for FMBIO Mac data generation
•Kermit Channel & Mary Robnett (AR State Laboratory) for FMBIO NT data generation

The many forensic scientists and their supervisors who took time out of their busy schedules to examine the MIX05 data provided as part of this interlaboratory study

Disclaimer

This project was funded by the National Institute of Justice through interagency agreement 2003-IJ-R-029 to the NIST Office of Law Enforcement Standards. Points of view are those of the authors and do not necessarily represent the official position or policies of the US Department of Justice. Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments, or equipment identified are necessarily the best available for the purpose.

For more information on MIX05, see STRBase:
http://www.cstl.nist.gov/biotech/strbase/interlab/MIX05.htm

•"If you show 10 colleagues a mixture, you will probably end up with 10 different answers"
–Peter Gill, Human Identification E-Symposium, April 14, 2005 (see http://www.humid.e-symposium.com/)